EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Consumer Products

SCCP

OPINION ON

1,3-bis-(2,4-Diaminophenoxy)propane

COLIPA n° A79

Scientific Committees
on consumer products
on emerging and newly identified health risks
on health and environmental risk

The SCCP adopted this opinion at its 12th plenary meeting on 19 June 2007

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCP

Questions concerning the safety of consumer products (non-food products intended for the consumer).
In particular, the Committee addresses questions related to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents and consumer services such as tattooing.

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Opinion to be cited as: Opinion of the SCCP on 1,3-bis-(2,4-diaminophenoxy)propane, 19 June 2007

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1. BACKGROUND

Submission I of 1,3-Bis-(2,4-diaminophenoxy)propane (A079) was submitted by COLIPA [1] in April 1988 according to COLIPA. The Scientific Committee on Cosmetology (SCC) concluded in its opinion from 10 December 1993 that:

"In absence of carcinogenicity data, the SCC requires an in vitro cytogenic study and an in vivo UDS study".

Submission II of 1, 3-Bis-(2,4-diaminophenoxy)propane was submitted by COLIPA in November 1995 according to COLIPA. The Scientific Committee on Cosmetics and Non-Food Products expressed at the plenary session of 23 June 1999 its opinion (SCCNFP/0136/99). SCCNFP concluded that:

"Classification: 1 under the conditions in use: in oxidative hair dyes at a maximum concentration of 2.0%, in combination with hydrogen peroxide the maximum use concentration upon application is 1.0%."

The substance is currently regulated by the Cosmetics Directive (76/768/EC), Annex III, Part 2 under entry 18 on the List of substances provisionally allowed, which cosmetic products must not contain except subject to restrictions and conditions laid down.

Submission III of 1,3-Bis-(2,4-diaminophenoxy)propane hydrochloride was submitted by COLIPA in July 2005. According to this submission the substance is used as a precursor for hair colours. It reacts with primary intermediates to form the final dye-stuff. The reaction can be accelerated by addition of an oxidizing agent (e.g. hydrogen peroxide), but can also be achieved by air oxidation. The final concentration on head can be up to 1.8% (calculated as tetrahydrochloride salt, corresponding to 1.2% of the free base). Under intended use conditions the exposure is terminated thirty minutes after application of the mixture to the hair by shampooing and thoroughly rinsing with water.

Submission III presents updated scientific data on the above mentioned substance in line with the second step of the strategy for the evaluation of hair dyes (http://europa.eu.int/comm/enterprise/cosmetics/doc/hairdyestrategyinternet.pdf) within the framework of the Cosmetics Directive 76/768/EEC.

2. TERMS OF REFERENCE

1. *Does the Scientific Committee on Consumer Products (SCCP) consider 1,3-Bis-(2,4-diaminophenoxy)propane and its tetra hydrochloride salt safe for use as non-oxidative hair dye formulations with a concentration of maximum 1.8 % (calculated as tetrahydrochloride salt, corresponding to 1.2 % of the free base) on the head taken into account the scientific data provided?*

2. *Does the SCCP consider 1,3-Bis-(2,4-diaminophenoxy)propane and its tetra hydro chloride salt safe for use as oxidative hair dye formulations with a final concentration 1.8 % on the head (calculated as tetrahydrochloride salt, corresponding to 1.2 % of the free base) taken into account the scientific data provided?*

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association

3. Does the SCCP recommend any restrictions with regard to the use of 1,3-Bis-(2,4-diaminophenoxy)propane and its tetrahydrochloride salt in oxidative or non-oxidative hair dye formulations (e.g. max conc. in the finish cosmetic product, dilution ratio with hydrogen peroxide etc.) beyond the existing allergenic warning?

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

1,3-bis-(2,4-Diaminophenoxy)propane (INCI)
1,3-bis-(2,4-Diaminophenoxy)propane HCl (INCI)

3.1.1.2. Chemical names

Free base
1,3-Benzenediamine, 4,4'-[1,3-propanediylbis(oxy)]bis- (9CI)

Tetrahydrochloride
1,3-Benzenediamine, 4,4'-[1,3-propanediylbis(oxy)]bis-, tetrahydrochloride (9CI)

3.1.1.3. Trade names and abbreviations

Free base
1,3-Di(2,4-diaminophenoxy)propane
1,3-Bis(2,4-diaminophenoxy)propane

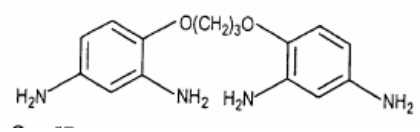
Tetrahydrochloride
3-Amino-4-[3-(,4-diaminophenoxy)propoxy]phenylamine tetrahydrochloride
1,3-Bis(2,4-diaminophenoxy)propane tetrahydrochloride
Ro 463
HC Blue 16

3.1.1.4. CAS / EINECS number

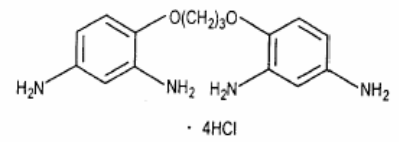
CAS: 81892-72-0 (free base)
74918-21-1(tetrahydrochloride)

EINECS: 279-845-4 (free base)
278-022-7 (tetrahydrochloride)

3.1.1.5. Structural formula



Free base



Tetrahydrochloride

3.1.1.6. Empirical formula

Formula: $C_{15}H_{20}N_4O_2$ (free base)
$C_{15}H_{20}N_4O_2$. 4 HCl (tetrahydrochloride)

3.1.2. Physical form

Grey powder

3.1.3. Molecular weight

Molecular weight: 288.35 (free base)
434.19 (tetrahydrochloride)

3.1.4. Purity, composition and substance codes

Data for the tetrahydrochloride:

Description		Batch number	
		7392	Ro-EI 5040/153
Titre NMR HPLC	 (%) (%)	 94.0 99.8	 93.5 ± 5 -
Impurities (HPLC) 3-(2,4-diaminophenoxy)propan-1-ol	 (%)	 1.2	 -
Ash content	(%)	0.8	-
Water content	(%)	4.6	-

3.1.5. Impurities / accompanying contaminants

No additional data

3.1.6. Solubility

Data for the tetrahydrochloride:

Water:	>100 g/l	room temperature
Ethanol:	< 1g/l	room temperature
DMSO:	10 – 100 g/l	room temperature

3.1.7. Partition coefficient (Log P_{ow})

n-octanol/water partition coefficient calculated (ACD) for the tetrahydrochloride:
Log P_{ow}: -1.54+/- 0.36

3.1.8. Additional physical and chemical specifications

Data for the tetrahydrochloride

Melting point:	/
Boiling point:	/
Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
pH:	/
UV_Vis spectrum	/

3.1.9. Stability

Standard solutions (1012 mg/l and 1036 mg/l) of the test substance in 30/70 acetonitrile/Milli-Q water were stable at least 42.8 hours when stored at room temperature in the dark.

General Comments to physico-chemical characterisation

* The batches of A 079 used in the acute oral toxicity test (Ro 463), in the prenatal developmental toxicity study (batch: Ro-El 5040/153) and in the i*n vivo* unscheduled DNA synthesis (UDS) test (batch: Ro-El 5040/153) are not fully analytically described.
* No data on stability in the test solutions and in the marketed product was provided.
* Log P_{ow}: calculated values cannot be accepted as estimates of the true physical constant without justification, indicating that the reported values are realistic.
* Data on physico-chemical properties of the test substance, including UV_Vis spectrum, are only limited.

3.2. Function and uses

1,3-bis-(2,4-Diaminophenoxy)-propane HCl is used as a precursor for hair colours. It reacts with primary intermediates to form the final dye-stuff. The reaction can be accelerated by addition of an oxidizing agent (e.g. hydrogen peroxide), but can also be achieved by air oxidation.

The final concentration of 1,3-bis-(2,4-diaminophenoxy)-propane HCl on head can be up to 1.8% (calculated as tetrahydrochloride salt, corresponding to 1.2% of the free base).

3.3. Toxicological Evaluation

All tests were carried out with the tetrahydrochloride salt.

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline: /
Species/strain: Wistar rats
Group size: 10 males per group
Test substance: Ro 463 (1,3-bis-(2,4-diaminophenoxy)-propane tetrahydrochloride)
Batch: /
Purity: /
Dose: 2510, 3160, 3570, 3980 and 5010 mg/kg bw
Observation period: 14 days
GLP: /

The acute oral toxicity of the test substance (tetrahydrochloride salt) was investigated in healthy young male rats of the Wistar strain. Fifty rats (10 rats per dose group) were used. At the beginning of the study the average body weight of the rats was 160 g. The test compound was dissolved in deionised water. Aliquots of 20 ml/kg bw were administered orally by gavage. The applied doses were 2510, 3160, 3570, 3980 and 5010 mg/kg bw, respectively. During a 14 day observation period, mortalities and clinical-toxicological observations were recorded daily.

Results
Lethality incidences at the end of the observation period were as follows: 0/10 (2510 mg/kg bw), 2/10 (3160 mg/kg bw), 5/10 (3570 mg/kg bw), 8/10 (3980 mg/kg bw), 9/10 (5010 mg/kg bw). The median acute lethal dose in male rats, demonstrated by the LD_{50}-value, was calculated to be 3570 mg/kg bw.

Ref.: 5

Comment
The study was not conducted according to GLP/OECD guideline. The major shortcomings were limited information of the test substance and that only one gender was used. However, the SCCP regards this study as sufficiently valid to avoid a repetition of the acute oral toxicity test.

3.3.1.2. Acute dermal toxicity

No data submitted

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline: OECD 404 (2002)
Species/strain: Albino Rabbit New Zealand White (SPF)
Group size: 3 males

Test substance:	A 079/ SAT 030632 (1,3-bis-(2,4-diaminophenoxy)-propane tetrahydrochloride)
Batch:	7392
Purity:	99.8%
Dose:	0.5 g under semi-occlusive conditions
GLP:	in compliance

An aliquot of 0.5 g of the moistened test substance was exposed to the intact shaved back skin of each animal. The patch was removed four hours after semi-occlusive contact. Animals were examined for signs of erythema, eschar and oedema formation. The skin reactions were assessed approximately 1 hour, 24, 48 and 72 hours after termination of the exposure and the effects were scored according to the OECD guideline.

Results
Under the conditions of the study, exposure to the undiluted test substance resulted in slight erythema in the treated skin areas of the three rabbits at one hour after treatment. No oedemas were observed. There was no evidence of a corrosive effect on the skin. The animals did not show any symptoms of systemic intoxication and no mortality occurred.

Conclusion
Under the conditions of the study, the neat test substance, applied under semi-occlusive conditions, showed minimal and transient irritancy to the skin.

Ref.: 6

3.3.2.2. Mucous membrane irritation

Guideline:	OECD 405 (2002)
Species/strain:	Albino Rabbit New Zealand White (SPF)
Group size:	3 males
Test substance:	A 079 / SAT 030632, (1,3-bis-(2,4-diaminophenoxy)-propane tetrahydrochloride)
Batch:	7392
Purity:	99.8%
Dose:	approximately 45 mg
GLP:	in compliance

Test substance (44.1 – 45.2 mg powder, volume approximately 0.1 ml) was instilled into the conjunctival sac of one eye of the test animals. The substance remained in permanent contact with the eyes until rinsing with warm tap water, 24 hours after instillation. The other eyes served as controls. The eye irritation reactions were scored 1 hour, 24, 48 and 72 hours as well as 7 (3 animals) and 14 days (two animals) after instillation of the test solution.

Results
The instillation of the undiluted test substance into the eyes resulted in effects on the cornea, iris and conjunctivae. The corneal injury consisted of opacity (maximum grade 1 in all animals up to 72 h after instillation) and epithelial damage (maximum 65% of the corneal area 24 h after instillation). The corneal injury had resolved within 72 hours or 7 days in the respective animals. Iridial irritation grade 1 was observed in all animals during the observation period and had resolved after 24 or 48 hours, or 7 days. The irritation of the conjunctivae consisted of redness (up to grade 3), chemosis (up to grade 4) and discharge (up to grade 2) and had completely resolved within 7 days in one animal and within 14 days in the other animals. The animals did not show any symptoms of systemic intoxication, no death occurred. Grey staining on head, fur and paws of all animals was noted one hour after instillation.

Conclusion
Under the conditions of the study, the test substance was irritating to rabbit eyes.

Ref.: 7

3.3.3. Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline:	OECD 429 (2002)
Species/strain:	CBA/J mice
Group size:	5 females / group
Test substance:	A 079/SAT 030632 (1,3-bis-(2,4-diaminophenoxy)-propane tetrahydrochloride)
Batch:	7392
Purity:	99.8%
Concentration:	5, 25 and 50% dye (w/v) in ethanol/water mixture (7/3, v/v)
GLP:	in compliance

Three dose groups and a control group (receiving the vehicle only) of 5 female mice each were investigated. A homogenous dilution of the test item in a mixture of ethanol:water (7:3 v/v) was made shortly before each dosing. The highest non-irritating concentration was found in a pre-test with four female mice. Based on these test results 5%, 25% and 50% solutions were chosen for the main study. The application volume of 25 µl was spread over the entire dorsal surface of each ear lobe once daily for three consecutive days. The control group was treated with the vehicle exclusively. Five days after the first topical application, all mice were administered with radio-labelled thymidine (^{3}HTdR) by intravenous injection via the tail vein. Approximately five hours after ^{3}HTdR application all mice were euthanized. The draining lymph nodes were excised and pooled for each experimental group. After preparation of the lymph nodes, the level of ^{3}HTdR incorporation was measured by liquid scintillation counting. The values obtained were used to calculate stimulation indices (SI). An appropriate reference (α-hexylcinnamic aldehyde) was used as positive control, to show distinct increases in SI. The proliferative capacity of pooled lymph node cells was determined by quantifying the incorporation of ^{3}H-TdR. A test item is regarded as a sensitizer if the exposure to at least one concentration resulted in an at least 3-fold increase in incorporation of ^{3}HTdR compared with concurrent controls, as indicated by the stimulation index (S.I.).

Results
Even at the highest concentration no skin effects were noted on the ear dorsum of the treated mice. The S.I. for the different dose groups were 1.2 ± 1.0 (5%), 4.9 ± 1.1 (25%) and 4.3 ± 1.0 (50%), respectively and 1.0 for the vehicle control. Data showed a dose-response and an EC3 value of 14.7% was calculated.

Conclusion
Based on the criteria of the test system, the test substance was found to be a moderate skin sensitizer in mice.

Ref.: 8

3.3.4. Dermal / percutaneous absorption

Guideline:	/
Species:	pig (two suckling pigs aged 6-8 weeks, sex not given)
Tissue:	skin from trunk area, dermatomed 400 µm
Groups:	eight replicates for formulation A and solution C, seven replicates for formulation B
Test substance:	unlabelled: A079 (1,3-bis-(2,4-diaminophenoxy)-propane tetrahydrochloride)

	Radiolabelled: A079 (1,3-bis-(2,4-diaminophenoxy)-propane tetrahydrochloride, [14C]- A 079)
Batch:	SAT 030632 (unlabelled) SAT 030760 (radiolabelled) SAT 030774 (OS3 developer mix with hydrogen peroxide) SAT 030775 (OS3 developer mix without hydrogen peroxide) SAT 030778 (OS7 cream formulation)
Purity:	SAT 030632 (unlabelled): 99.8%
Dose levels:	final nominal concentrations of 1.5% (w/w) in cream formulation with (A) and without hydrogen peroxide (B) and in an aqueous solution (C)
Exposure time:	30 minutes
GLP:	in compliance

The dermal absorption/percutaneous penetration of [14C]-1,3-bis-(2,4-Diaminophenoxy)propane out of a basic cream (mixed with a developer with and without hydrogen peroxide) and from an aqueous solution was studied on the clipped excised skin of suckling pigs (aged 6-8 weeks). The integrity of the skin discs was checked by measuring the trans-dermal electrical resistance. The intact, clipped excised pig skin of the flanks area was exposed for 30 minutes to the test substance in the basic hair dyeing formulations/solution without occlusion (10 mg/cm²). Glass diffusion cells were used with an exposed membrane area of 2.54 cm^2. Each of the two formulations and the solution were analysed with eight replicates for adsorbed, absorbed and penetrated amount of the test substance. Samples of receptor fluid were taken at recorded intervals (Pre, 0.5, 2, 4, 6, 12, 24, 30, 36 and 48 h). The receptor fluid used was physiological buffered saline. After the 0.5 h sample the skin was washed. For mass balancing samples of receptor fluid, remaining receptor fluid at the end of experiment, washes, successive layers of the stratum corneum (21 tape strippings) and the epidermis and dermis were measured.

Results
The quantities of test substance detected are shown in the following table. Both the amounts measured in epidermis and dermis as well as amounts in the receptor fluid were taken as systemically available. Mean values for percutaneous absorption are 1.34 ± 0.697 µg/cm^2 (formulation A, with H_2O_2), 1.696 ± 0.682 µg/cm^2 (formulation B, without H_2O_2), 0.670 ± 0.635 µg/cm^2 (aqueous solution).

ANALYSED SAMPLE	Formulation A with H_2O_2 [µg/cm²] [%]	Formulation B without H_2O_2 [µg/cm²] [%]	Solution C [µg/cm²] [%]
Skin rinsings	159.26 – 204.26 90.34 – 116.06	129.21 – 177.78 71.78 – 98.77	165.16 – 185.50 93.84 – 105.34
Adsorption (stratum corneum)	0.090 – 0.181 0.05 – 0.103	0.090 – 0.183 0.05 – 0.102	0.151 – 0.509 0.086 – 0.289
Not Bio-available	**160.661 – 204.547** **91.29 – 116.22**	**129.503 - 178.171** **71.95 – 98.98**	**165.803 – 187.070** **94.21 – 106.29**
Absorption (epidermis/dermis)	0.306 – 2.26 0.174 – 1.284	1.09 – 2.83 0.606 – 1.572	0.128 – 2.04 0.073 – 1.159
Penetration (receptor fluid)	0.018 – 0.051 0.010 – 0.029	0.015 – 0.017 0.008 – 0.009	0.013 – 0.025 0.007 – 0.014
Bio-available	**0.333 – 2.278** **0.189 – 1.294**	**1.106 – 2.846** **0.614 – 1.581**	**0.150 – 2.054** **0.085 – 1.167**
Total recovery / mass balance	**91.4 - 117**	**72.8 – 99.5**	**95.3 - 106**

Ref.: 17

Comment
The study was approximated to the opinion on *basic criteria for the in vitro assessment of dermal absorption of cosmetic ingredients* from the SCCP, however only two instead of three donors (8 and 7 replicates per group) were used for the skin samples. No justification

for the use of static conditions is given. The recovery was > 115% in one sample (formulation A with H_2O_2) and < 85% in one sample (formulation B, without H_2O_2). The concentration of 1,3-bis-(2,4-diaminophenoxy)-propane tetrahydrochloride used in the study was 1.5% while the requested concentration is 1.8%. Despite these shortcomings the test is valid for risk evaluation.

The mean values plus 2 standard deviations values of the bio-available amount found for formulation A (2.73 µg/cm^2) and formulation B (3.06 µg/cm^2) were used for MOS calculation.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

No data submitted

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

Guideline:	OECD 408 (1998)
Species/strain:	Wistar strain (Crl:(WI)BR)
Group size:	12 animals per sex and group
Test substance:	A 079, (1,3-bis-(2,4-diaminophenoxy)-propane tetrahydrochloride)
Batch number:	7392
Purity:	99.8%
Dose levels:	0, 40, 120 and 360 mg/kg bw/day in water (10 ml/kg) by gavage
Exposure period:	13 weeks
GLP:	in compliance

The test substance was given daily as an aqueous solution for a period of 13 consecutive weeks in doses of 0, 40, 120 and 360 mg/kg bw/day based on the results of a dose range finding study (0, 50, 450 and 1000 mg/kg bw/day). Distilled water was used as an appropriate vehicle for this test substance. Additionally 10 rats (5 per sex) for both the control and the high dose group were assessed for recovery of treatment-related effects, four weeks after the last administration. During the study mortality, signs of intoxication, body weight and food consumption were recorded. The animals of the recovery groups were additionally examined during the 4-week treatment-free period. At the end of the study, the animals were sacrificed and subjected to pathological investigations.

Results
Brown discolouration/staining of various body parts and brown discolouration of the urine and grey/blue discolouration of the mouth, snout and/or tongue at 360 mg/kg bw/day noted during the treatment phase were considered to be due to the systemic presence of the test substance. This was corroborated by the presence of brown pigment in most organs at all dose groups and black discolouration of various organs recorded at necropsy. These findings remained present during the recovery phase.

Dark appearance of the eyes recorded in all animals at 120 and 360 mg/kg bw/day occurred in conjunction with multifocal circumscribed retinopathy (uni- or bilateral) and trembling of the eyeball in a few high dose animals at the end of treatment. Blue discolouration of the sclera confirmed the dark appearance of the eyes of high dose animals at the end of recovery. It could not be excluded that the histological appearance of brown pigment (considered to be the test substance) in the choroid of the eyes was directly related to the ophthalmologic effects noted. Pupillary reflex tests at the end of treatment revealed no abnormalities.

Slightly reduced weight gain was noted for high dose males during the treatment phase. Absolute body weight of these animals remained reduced during the recovery phase. Additionally, body weights of high dose females were slightly lower during recovery phase. There were no changes in food consumption in these groups considered to be correlated to the test substance.

Several effects related to the test substance were reported for the 120 mg/kg and the 360 mg/kg dose groups like lower thymus and adrenal weights, increased spleen weights, higher kidney weights in combination with lower inorganic phosphate and chloride levels, and higher albumin and urea levels as well as higher liver weights in combination with an increased incidence of inflammatory cell foci in the liver, intimal proliferation of the large veins, lower total protein levels, and increased cholesterol and alkaline phosphatase activity levels. Minor degenerative changes in the heart (degeneration and regeneration with an inflammatory reaction, in some cases with fibrosis) and oesophagus (degeneration of the muscle layer with vacuolation of the fibres) at 360 mg/kg bw/day, and cardial myopathy in some animals at 120 mg/kg bw/day, occurred in conjunction with higher aspartate aminotransferase activity in males and females at 360 mg/kg bw/day. Alterations in the nervous system occurred at low incidences in a few males at 120 and 360 mg/kg bw/day, and consisted of vacuolation/degeneration of the white matter of the brain and spinal cord and of the myelin sheet of the sciatic nerve. Most of the effects were still evident at the end of the recovery period. Several changes in haematological and biochemical parameters were reported which had resolved at the end of the recovery period.

No cause of death was established microscopically for the two females at 120 and 360 mg/kg bw/day respectively that did not survive until scheduled necropsy. Based on the absence of supportive clinical signs or further mortality in these dose groups, these deaths were considered to be of an incidental nature. Salivation was noted among all dose groups at several observation periods during treatment.

Changes in red blood cell parameters at 40 mg/kg bw/day (i.e. increased mean corpuscular volume and red cell distribution width) were reported to be within the normal physiological range, and for the red cell distribution width the control value was considered to be rather low. There was no evidence of organ dysfunction. Renal tubular basophilia and hyaline casts were noted in both sexes at 40 mg/kg bw/day and reported to be of a minor degree with regard to both incidence and severity and were not corroborated by any disturbances indicative of an adverse effect on body functions at this dose level. Therefore, the No Observed Adverse Effect Level (NOAEL) for 1,3-bis-(2,4-Diaminophenoxy)propane was established at 40 mg/kg bw/day.

Ref.: 14

Comment
Although there were no organ dysfunctions reported, effects on haematological and biochemical parameters reported for males in the 40 mg/kg dose group might be related to the test substance. The increase in mean corpuscular volume (MCV) and the decrease of alkaline phosphatase (ALP) were dose dependent. In addition, variations in mean corpuscular haemoglobin concentration (MCHC) values and the decrease of ALP were reported for males of the highest dose group at the end of the recovery period. Together with the occurrence of renal tubular basophilia and hyaline casts, these effects might be first indicators for organ toxicity, especially since the kidney is one of the target organs in the higher dose groups. Therefore a Lowest Observed Effect Level (LOEL) was established by the SCCP at 40 mg/kg bw/day equivalent to 26 mg/kg bw per day of the free base.

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial gene mutation assay

Guidelines:	OECD 471 (1997)
Species/strain:	*Salmonella typhimurium* TA98, TA100, TA102, TA1535, TA1537 Replicates: triplicates, two independent tests
Test substance:	A 079 (1,3-bis-(2,4-diaminophenoxy)-propane tetrahydrochloride
Batch:	7392
Purity:	99.8%
Concentrations:	33, 100, 333, 1000, 2500, 5000 µg dye/plate, with and without metabolic activation
GLP:	in compliance

The Ames-test was performed with the bacterial tester strains *Salmonella typhimurium* TA98, 100, 102, 1535 and 1537 with and without phenobarbital and β-naphthoflavone induced rat liver enzymes (S9-mix). Test substance was tested in deionised water at six concentrations in the range of 33 to 5000 µg/plate. This range based upon the results of the pre-experiment. The assay was performed in two independent experiments both with and without liver microsomal activation. Sodium azide (10 µg/plate) served as a positive control for TA100 and TA1535, 4-nitro-o-phenylene-diamine (10 µg/plate) for TA1537 and TA98 and methyl methane sulfonate (4 µg/plate) for TA102 without S9-mix. The enzyme activity of S9-mix was separately controlled by testing 2-aminoanthracene (2.5 µg/plate) in all tester strains. The solvent deionised water and the untreated fresh cell suspension served as negative controls.

Results
The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without S9-mix in all strains used. No reduction in the number of revertants, was observed with and without metabolic activation in all strains used. No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with 1,3-bis-(2,4-Diaminophenoxy)propane HCl at any dose level, neither in the presence nor absence of metabolic activation. Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

Conclusion
1,3-bis-(2,4-Diaminophenoxy)propane HCl is considered to be non-mutagenic in this *in vitro* bacterial mutagenicity test in the presence or absence of S9-mix.

Ref.: 9

In Vitro* Mouse Lymphoma gene mutation assay (*tk* locus*)

Guideline:	OECD 476 (1997)
Species/strain:	Mouse lymphoma cell line L5178Y/TK$^{+/-}$
Replicates:	Duplicates, two independent tests
Test substance:	A 079 (1,3-bis-(2,4-diaminophenoxy)-propane tetrahydrochloride)
Batch:	7392
Purity:	99.8%
Concentrations:	68.8, 137.5, 275.0, 412.5 and 550.0 µg/ml without metabolic activation (experiment 1) 68.8, 137.5, 275.0, 550.0 and 1100.0 µg/ml with metabolic activation (experiment 1)

	9.4, 18.8, 37.5, 75.0, 112.5, 150.0 and 300.0 µg/ml; without metabolic activation (experiment 2)
Treatment time:	4 h (experiment 1) 24 h (experiment 2)
GLP:	in compliance

The assay was performed in two independent experiments, using two parallel cultures each in the presence and absence of phenobarbital and β-naphthoflavone stimulated rat liver S9-mix. The first main experiment was performed with a treatment period of 4 h with and without metabolic activation. The second experiment was solely performed in the absence of metabolic activation with a treatment period of 24 hours. The test substance was dissolved in deionised water and, according to the results of the pre-test, adequate concentrations were chosen for the mutation experiment. The cultures at 150 and 300 µg/ml in experiment II were not continued due to severe toxic effects. Appropriate references (methyl methane sulfonate 13 µg/ml without S9-mix and cyclophosphamide 4.5 µg/ml with S9-mix) were used as positive controls.

Results
Precipitation was exclusively observed at 300 µg/ml in the second experiment without metabolic activation. Appropriate levels of cytotoxicity (10-20% survival at the highest dose tested) were not reached in experiments without S9-mix. Without metabolic activation the test substance induced strong toxic effects in both parallel cultures at 412.5 µg/ml and above (experiment 1) and from 75 µg/ml and above (experiment 2) indicated by a relative total growth < 10% of controls. Values for these concentrations, therefore, were not considered as valid. Moderate toxicity (above the appropriate levels of cytotoxicity for the highest analysable dose) was observed at 275 µg/ml (experiment 1) and 37.5 µg/ml (experiment 2). In the presence of metabolic activation the appropriate level of cytotoxicity was reached in 1 of the 2 cultures at the highest dose of 1100 µg/ml.
In both main experiments no relevant and reproducible increase of the mutation frequency was observed. The threshold of twice the mutation frequency of the corresponding solvent control was reached or exceeded at 68.8 and 275 µg/ml in the second culture of experiment 1 without metabolic activation. This increase however, was not reproduced in the first culture under identical conditions and is interpreted as biologically irrelevant fluctuation. Furthermore, the absolute values of the mutation frequency remained well within the historical range of negative and solvent controls. Appropriate reference mutagens were used as positive controls and showed a distinct increase in induced total mutant colonies and an increase of the relative quantity of small versus large colonies.

Conclusion
Under the experimental conditions reported, 1,3-bis-(2,4-Diaminophenoxy)propane HCl did not induce mutations in this gene mutation tests in mammalian cells neither in the absence nor presence of metabolic activation.

Ref.: 10

In vitro chromosome aberration test

Guideline:	OECD 473
Species/strain:	Chinese Hamster Ovary (CHO Cells)
Replicates:	Duplicates
Test substance:	A 079 (1,3-bis-(2,4-diaminophenoxy)-propane tetrahydrochloride)
Batch:	7392
Purity:	99.8%
Concentrations:	300, 400 and 500 µg/ml without metabolic activation 800, 1000 and 1200 µg/ml with metabolic activation
Exposure period:	4 hours
GLP:	in compliance

The chromosome aberration assay was performed in the presence and absence of phenobarbital and β-naphthoflavone stimulated rat liver S9-mix. The chromosomes were prepared 18 hours after start of treatment. The treatment interval was four hours with and without metabolic activation. 100 metaphases per culture were scored for structural chromosome aberrations. Appropriate references (ethyl methane sulfate 200 µg/ml without S9-mix and cyclophosphamide 0.7 µg/ml with S9-mix) were used as positive controls to show distinct increases in cells with structural chromosome aberrations.

Results
In a pre-test on toxicity toxic effects indicated by reduced mitotic indices of below 50% of control were observed at 543.8 µg/ml (without metabolic activation) and 1087.5 µg/ml (with metabolic activation). In the main experiment in the presence of S9-mix the required reduction of the mitotic index by 50% was not reached. Both, in the absence and in the presence of S9-mix, statistically significant and biologically relevant concentration-dependent increases in the number of cells carrying structural chromosomal aberrations were observed after treatment with the test substance. In the presence of S9-mix an increase in the number of polyploid metaphases was observed exceeding the historical control data range at all concentrations evaluated. In addition, the number of endomitotic metaphases was distinctly increased. The positive controls used induced statistically significant increases in cells with structural chromosome aberrations.

Conclusion
Under the experimental conditions, 1,3-bis-(2,4-Diaminophenoxy)propane HCl induced structural (without and with S9-mix) and numerical (with S9-mix) chromosome aberrations in the V79-cell line and was found to be clastogenic and possibly aneugenic in this *in vitro* assay.

Ref.: 11

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Mouse bone marrow micronucleus test

Guideline:	OECD 474
Species:	NMRI mice
Group sizes:	6 animals/sex/dose/killing time
Test substance:	A 079, SAT 030384 (1,3-bis-(2,4-diaminophenoxy)-propane tetrahydrochloride)
Batch:	7392
Purity:	99.8%
Dose levels:	0, 62.5, 125 and 250 mg/kg bw in deionised water, 10 ml/kg (100, 200, 250, 300 mg/kg bw in pre-experiment on toxicity)
Exposure route:	i.p.
GLP:	in compliance

1,3-bis-(2,4-Diaminophenoxy)propane HCl, dissolved in deionised water was administered intraperitoneally in a single dose of 62.5, 125 and 250 mg/kg bw (10 ml/kg). Six NMRI mice were used per dose and sex. Additional 16 animals (two per dose and sex) had been used in pre-experiments for toxicity and received doses of 100, 200, 250 or 300 mg/kg bw as single i.p. injection. Bone marrow of femurs was prepared 24 and 48 (only for the high dose level) hours after application of the test substance. Cyclophosphamide monohydrate (40 mg/kg bw) and the vehicle, respectively served as positive and negative controls.
For each animal at least 2,000 polychromatic erythrocytes (PCE) obtained from femoral bone marrow were examined. The frequency of micronuclei was calculated for each animal and dose group.

Results
After treatment with the test substance the number of PCEs was not substantially decreased as compared to the mean value of PCEs of the vehicle control and, therefore, did not indicate a cytotoxic effect of 1,3-bis-(2,4-Diaminophenoxy)propane HCl in the bone marrow. The bio-availability of the test item was, however, confirmed by chemical analysis of the blood of the treated animals. In the pre-experiment on toxicity one of the males treated with 300 mg/kg bw died within 48 h after application of the test substance.
The positive control substance caused cytotoxicity and produced micronuclei in polychromatic erythrocytes, thus demonstrating the sensitivity of the test system used for the endpoints investigated in this study. The test substance did not induce an increase in the number of cells with micronuclei.

Conclusion
From the results obtained in this study, it was concluded that 1,3-bis-(2,4-Diaminophenoxy)propane HCl did not show any evidence of mutagenic (clastogenic and/or aneugenic) potential in this *in vivo* test for chromosomal alterations when administered intraperitoneally to mice.

Ref.: 12

In vivo unscheduled DNA synthesis (UDS) test

Guideline:	/
Species:	Wistar rats (CF HB strain)
Group sizes:	3 males per dose level
Test substance:	Ro 463 (1,3-bis-(2,4-diaminophenoxy)-propane tetrahydrochloride
Batch no:	Ro-El 5040/153
Purity:	approximately 95%
Dose levels:	1,000 and 2,000 mg/kg in distilled water
Exposure route:	oral (gavage)
GLP:	in compliance

Four male Wistar rats per dose aged 8 to 10 weeks at the time of treatment were used in the experiment. Vehicle only, the test substance or the positive control substance (2-acetylaminofluorene in corn oil and DMSO) at a dosage-volume of 10 ml/kg, respectively 8 mg/kg for the control substance were applied orally. Two independent experiments were performed. Liver perfusions were undertaken for three animals per group approximately fourteen hours after treatment in the first experiment and approximately two hours after treatment in the second experiment. Primary rat hepatocytes were prepared by perfusing rat liver *in situ*. Ninety minutes after plating, the cells were treated with [^{3}H]-thymidine for a period of four hours. A total of 100 cells from each animal were scored. Nuclear and cytoplasmic grains were counted separately and the cytoplasmic counts were subtracted from the nuclear counts to give net grain / nucleus (NG).

Results
Treatment with the test substance did not increase NGs at any treatment level in either assay. Marked increases in UDS were observed following treatment with the positive control compound.

Conclusion
1,3-bis-(2,4-Diaminophenoxy)propane HCl did not induce unscheduled DNA synthesis in primary rat hepatocytes after *in vivo* treatment under the reported experimental conditions.

Ref.: 13

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Embryotoxicity/Teratogenicity

Guideline:	/
Species/strain:	Sprague Dawley rats
Group size:	21 - 24 females
Test substance:	Ro 463 (tetrahydrochloride salt)
Batch number:	Ro-El 4050/153 (1,3-bis-(2,4-diaminophenoxy)-propane tetrahydrochloride)
Purity:	/
Dose levels:	0, 20, 60 and 180 mg /kg/day (gavage)
Treatment period:	day 6 - 15 of gestation
GLP:	/

Females (approx. 11 weeks of age) were paired with male rats of the same strain with an accurate day of mating (G0), fixed by the presence of vaginal plugs or sperm in the vaginal smear. Aliquots of 10 ml/kg bw of test substance at dose levels of 0, 20, 60 and 180 mg/kg bw, were administered daily (day 6-15 of pregnancy) by gavage. Distilled water was used as solvent and vehicle. Mortality and symptoms were observed twice daily. Body weight gain was determined on day 0, 6, 15 and 19 of gestation. The dams were sacrificed on day 20 post-coitum and subjected to necropsy. The number of alive and dead foetuses, their distribution and site in the uterus, early and late resorption, implantation and number of *corpora lutea* was determined. The weight of the foetuses, gravid uteri, uteri without foetuses, placentae and the sex of foetuses were recorded. Approximately one-third of the foetuses were selected at random and examined for visceral anomalies and variations. The remaining foetuses were examined for skeletal malformations, variations and retardation of the normal organogenesis after appropriate staining.

Results
No maternal deaths were observed during application. Apart from excretion of the coloured test substance or unspecified metabolites in the urine the dams were without any symptoms. Macroscopic examination of the inner organs of the dams did not result in any substance related findings. The median body weights in the substance treated groups were increased compared to the control group. This is explained by the low initial body weight of the animals in the control group. No indication of substance related influence on the outcome of pregnancy was found during the experiment. Pre-implantation litter data did not show any dosage-related trends. Resorption rates and post-implantation data were not adversely influenced by treatment. Number and sex ratio of the foetuses were comparable in all groups. No treatment related findings were observed in foetuses.

Conclusion
The No-Observed-Adverse-Effect-Level (NOAEL) for maternal and foetal toxicity is considered to be 180 mg/kg bw/day.

Ref.: 16

3.3.9. Toxicokinetics

No data submitted

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

No data submitted

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

(1,3-bis-(2,4-Diaminophenoxy)-propane HCl)
(Oxidative / permanent, calculated for the hydrochloride)

Standard oxidative conditions:

Maximum absorption through the skin	**A (µg/cm^2)**	**=**	**2.73 µg/cm²**
Skin Area surface	**SAS (cm^2)**	**=**	**700 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**1.911 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.032 mg/kg**
Lowest observed effect level (mg/kg) (subchronic oral toxicity study, rats)	**LOEL**	**=**	**40 mg/kg**

Provisional Margin of Safety	**LOEL / SED**	**=**	**1256**

Hair Dye Formulation without H_2O_2

Maximum absorption through the skin	**A (µg/cm^2)**	**=**	**3.06 µg/cm²**
Skin Area surface	**SAS (cm^2)**	**=**	**700 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**2.14 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.036 mg/kg**
Lowest observed effect level (mg/kg) (subchronic oral toxicity study, rats)	**LOEL**	**=**	**40 mg/kg**

Provisional Margin of Safety	**LOEL / SED**	**=**	**1120**

3.3.14. Discussion

Physico-chemical properties
Data on physico-chemical properties of the test substance, including UV_Vis spectrum, are only limited. The batches of 1,3-bis-(2,4-Diaminophenoxy)propane used in the acute oral toxicity test (Ro 463) and the prenatal developmental toxicity study (batch: Ro-El 5040/153) are not fully analytically described. No data on stability in the test solutions and in the marketed product was provided.

Percutaneous absorption
The study was approximated to the opinion on *basic criteria for the in vitro assessment of dermal absorption of cosmetic ingredients* from the SCCP, however only two instead of three donors were used for the skin samples. No justification for the use of static conditions is given. The recovery was > 115% in one sample (formulation A with H_2O_2) and < 85% in one sample (formulation B, without H_2O_2). The concentration of 1,3-bis-(2,4-diaminophenoxy)-propane tetrahydrochloride used in the study was 1.5% while the requested concentration is 1.8%. Despite these shortcomings the test is valid for risk evaluation. The mean values plus 2 standard deviations values of the bio-available amount found for formulation A (2.73 µg/cm^2) and formulation B (3.06 µg/cm^2) were used for MOS calculation.

General toxicity
In the subchronic oral toxicity study with rats the lowest dose tested (40 mg/kg) revealed changes of haematological and biochemical parameters mainly in males. The increase in mean corpuscular volume (MCV) and the decrease of ALP were dose depending, in addition variations in MCHC values and the decrease of ALP were reported for males of the highest dose group at the end of the recovery period. Together with the occurrence of renal tubular basophilia and hyaline casts these effects might be first indicators for organ toxicity, especially as the kidney is one of the target organs in the higher dose groups. Therefore a Lowest Observed Effect Level (LOEL) is established at 40 mg/kg bw/day for 1,3-bis-(2,4-Diaminophenoxy)propane HCl.
The use of the LOEL instead of the NOAEL is acceptable because of the high Margin of Safety calculated.

Irritation / sensitisation
Under the conditions of the study, the neat test substance, applied under semi-occlusive conditions, showed minimal and transient irritancy to the skin. It was irritating to mucous membranes. 1,3-bis-(2,4-Diaminophenoxy)propane was found to be sensitizing in the Local Lymph Node Assay (LLNA) with an EC3 value of 14.7% (moderate sensitiser).

Mutagenicity / genotoxicity
Overall, the three endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy were appropriately covered for 1,3-bis-(2,4-Diaminophenoxy)propane HCl. The test substance was not mutagenic *in vitro* when tested in a bacterial mutagenicity test in the presence or absence of S9-mix and did not induce mutations in the mouse lymphoma thymidine kinase locus assay in the absence and presence of metabolic activation.
1,3-bis-(2,4-Diaminophenoxy)propane HCl induced structural chromosome aberrations in the V79-cell line in the absence and presence of metabolic activation and was found to be clastogenic in this *in vitro* assay.
In *in vivo* tests, 1,3-bis-(2,4-Diaminophenoxy)propane HCl did not show any evidence of mutagenic potential for chromosomal alterations when administered intraperitoneally to mice and did not induce unscheduled DNA synthesis in primary rat hepatocytes.

Consequently, 1,3-bis-(2,4-Diaminophenoxy)propane HCl itself can be considered to have no relevant genotoxic potential *in vivo*. Additional tests are not necessary. To reach a definitive conclusion, appropriate tests with 1,5-naphthalenediol in combination with hydrogen peroxide have to be provided.

4. CONCLUSION

The SCCP is of the opinion that the use of 1,3-bis-(2,4-Diaminophenoxy)propane, and its tetrahydrochloride, itself as an oxidative and non-oxidative hair dye at a maximum concentration of 1.8 % (calculated as tetrahydrochloride salt, corresponding to 1.2 % of the free base) on the head does not pose a risk to the health of the consumer.

Beyond the existing allergenic warning, no further restrictions are deemed necessary.

However, studies on genotoxicity/mutagenicity in finished hair dye formulations should be undertaken following the relevant SCCNFP/SCCP opinions and in accordance with its Notes of Guidance.

5. MINORITY OPINION

Not applicable

6. REFERENCES

1. Meinigke, B. (2005). Raw material description – A 079. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0500035
2. Meinigke, B. (2005). Dossier of hair dye A079 – analysis of batch 7392 used in toxicological tests. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0500140
3. Opiela, E. and Sladek (1994). Analysis of identity and purity for a toxicological test – Ro 463. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0500141
4. Hollenberg, D. (2005) Dossier of hair dye A079 – analysis of batch Ro-El 5040/153 used in prenatal developmental toxicity study. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0500223
5. Potokar (1979). 1,3-Bis(2,4-diaminophenoxy)-propanetetrahydrochlorid = Ro 463, Prüfung der akuten oralen Toxizität an der Ratte, Institut für Toxikologie, Henkel KGaA, Düsseldorf, Germany, Archive code at Henkel KGaA, Düsseldorf, Report No. R 9400960
6. Otterdijk, F.M. (2004). Primary skin irritation / corrosion study with A 079 / SAT 030632 in the rabbit (4-hour semi-occlusive application). NOTOX B.V., s'-Hertogenbosch, The Netherlands, internal study code: 395764. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0400143
7. Otterdijk, F.M. (2004). Acute eye irritation / corrosion study with A 079 / SAT 030632 in the rabbit. NOTOX B.V., s'-Hertogenbosch, The Netherlands, internal study code: 395775. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0400133
8. van Huygevoort, A.H.B.M. (2004). Assessment of contact hypersensitivity to A 079 / SAT 030632 in the mouse (Local Lymph Node Assay). NOTOX B.V., 's-Hertogenbosch,

The Netherlands, internal study code: 398104. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0400345
9. Sokolowski, A. (2003). *Salmonella typhimurium* reverse mutation assay with A 079. RCC Cytotest Cell Research GmbH, Rossdorf, Germany, internal study code 800605. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0300658
10. Wollny, H.-E. (2005). Cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells with A 079. RCC Cytotest Cell Research GmbH, Rossdorf, Germany, internal study code 864503, Archive code at Henkel KGaA, Report No. R 0500137
11. Schulz, M. (2004). *In vitro* chromosome aberration assay in Chinese hamster V79 cells with A 079. RCC Cytotest Cell Research GmbH, Rossdorf, Germany, internal study code 800613. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0400197
12. Honarvar, N. (2004). Micronucleus assay in bone marrow cells of the mouse with A 079. RCC Cytotest Cell Research GmbH, Rossdorf, Germany, internal study code 838708. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0500233
13. Nunziata, A. (1994). Unscheduled DNA synthesis (UDS) in primary rat hepatocytes after in vivo treatment (autoradiographic method), test substance Ro 463. RTC Research Toxicology Centre S.p.A., Pomezia, Italy, RTC Report no.: 4210-M-06094. Archive code at Henkel KGaA, Düsseldorf, Report No. R 9400219
14. Otterdijk, F.M. (2001). Repeated dose 90-day oral toxicity study with A 079 by daily gavage in the rat followed by a 28-day recovery period. NOTOX B.V., 's-Hertogenbosch, The Netherlands, internal study code 409578. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0500175
15. Brands, C. (2004). Development and validation of an analytical method for the analysis of a A 079 in Milli-U water. NOTOX B.V., 's-Hertogenbosch, The Netherlands, internal study code 409591. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0400560
16. Potokar, M and Pittermann, W. (1987). 1,3-Bis-(2,4-diamino-phenoxy)-propan, Prüfung auf Embryotoxizität an Ratten. Institut für Toxikologie, Henkel KGaA, Düsseldorf, Germany. Archive code at Henkel KGaA, Düsseldorf, Report No. TBD 870345
17. Owen, H.M. (2004). A 079: *in vitro* penetration through pig skin from three different vehicles. Central Toxicology Laboratory, Cheshire, UK, internal Study No. JV1786. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0400276